

Surveillance of Enterovirus Infections in Iowa 1957-62

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IN JULY 1955, an extensive outbreak of an illness resembling nonparalytic poliomyelitis occurred in Marshalltown, Iowa (1). ECHO (enteric cytopathogenic human orphan) virus type 4 was associated with the illness (2). The following year several regional outbreaks of a similar illness occurred throughout the State (3, 4). Coxsackie virus B5 was found to be the etiological agent associated with these outbreaks (5).

The occurrence of major epidemics of viral illnesses during the next 2 years prompted the Kansas City (Kans.) Field Station, Communicable Disease Center, Public Health Service, cooperating with the Iowa State Department of Health, Des Moines, to conduct a study of the occurrence of enterovirus infections in the State of Iowa. The program, emphasizing the surveillance of poliomyelitis and poliomyelitis-like disease, was established in 1957.

During the surveillance period, 1957-62, two major outbreaks of enterovirus infection were observed. One was a large epidemic of poliomyelitis in Des Moines in 1959 (6). Our surveillance studies indicated that poliovirus infection was widespread throughout the State. The other was an outbreak of Coxsackie B5 virus infection in Fort Dodge in 1961.

Materials and Methods

Specimen collection. With the cooperation of the State health department, eight regional specimen-collection depots were established at

selected hospitals in eight different cities: Mason City, Waterloo, Fort Dodge, Ottumwa, Council Bluffs, Sioux City, Des Moines, and Iowa City. These depots were supervised by a pathologist or local health officer. Instructions and materials for collecting specimens were provided by Dr. W. J. Hausler, Jr., State hygienic laboratory, Iowa State Department of Health, and distributed from each depot.

Specimens of blood, stool, throat washings, and sometimes spinal fluid, were collected by physicians from patients with poliomyelitis and aseptic meningitis in different areas of the State and sent to the designated regional collecting depot. A form accompanying each specimen provided the patient's name, age, sex, residence, diagnosis, and date of onset of illness. The specimens were either taken to the depot or mailed in dry ice, and the depot periodically shipped accumulated frozen specimens to the laboratory at Kansas City.

Before 1959 the paralytic status of patients was based on information recorded on hospital charts. Beginning in 1959 the diagnosis of paralysis was based on examination made 60 days after onset of illness. Specimens for virological study were obtained from patients whose

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cases had been reported to the State epidemiologist and sometimes from patients whose cases had not been reported.

Laboratory reports. As soon as the virus isolation study of specimens from an individual patient provided a definitive result (negative, poliovirus isolated, or virus other than poliovirus isolated), the laboratory sent a report to the physician who submitted the specimens and to the State epidemiologist. Another report was mailed after the virus was identified. These reports informed the physicians of the occurrence of certain enteroviruses in their community and promoted their interest in the program. A list of the laboratory results obtained on specimens also was sent to the collaborating pathologist or health officer of the respective depots.

Specimen preparation. Specimens were prepared according to described methods (7). Ten percent stool suspensions were made in sterile demineralized water. Stool suspensions and turbid throat washings were centrifuged at 15,000 rpm for 30 minutes at 4° C. Penicillin (200 units), streptomycin (200 micrograms), and amphotericin B (1 microgram) were added to each milliliter of stool extract and throat washing.

Virus isolation and identification. Each specimen was tested in three primary monolayer cultures of monkey kidney cells with 0.1 ml. of inoculum per culture. The cultures were incubated at 37° C. and examined daily for 7 days. Cultures showing evidence of cytopathogenic changes were harvested by freezing and

stored at -20° C. Specimens causing no cytopathic changes in the cultures at the end of the observation period were considered to be negative. If toxic or nonspecific changes occurred in the cultures during the primary passage, an additional passage was made.

All the specimens showing cytopathic changes were tested by neutralization with specific hyperimmune rabbit serum against poliovirus types I, II, and III. Equal volumes of serum and infected-cell-culture fluid, diluted to contain about 200 tissue culture infective doses, were combined and incubated at 37° C. for 1 hour. Triplicate primary cultures of monkey kidney cells were inoculated with 0.1 ml. of the serum-virus mixture and incubated at 37° C. Diluted virus alone was inoculated as a positive control. A virus was considered to be neutralized by a specific serum when degeneration had not occurred in tubes inoculated with that serum-virus mixture 2 days after degeneration occurred in the positive control. Agents not neutralized with the poliovirus antisera were tested next with Coxsackie antisera and then ECHO antisera, using the method described.

Results

The annual distribution of cases reported to the State health department for the 6-year period 1957-62 is recorded in table 1. Of 78 cases reported in 1957, 21 were paralytic and 57 nonparalytic. The rate for nonparalytic cases was slightly more than 2.5 times that of paralytic cases. In 1958 a total of 73 cases was reported.

Table 1. Annual distribution of reported cases of poliomyelitis and poliomyelitis-like illness, Iowa, 1957-62

Year	Population ¹	Paralytic patients		Nonparalytic patients		Total	
		Number	Rate per 100,000	Number	Rate per 100,000	Number	Rate per 100,000
1957-----	2, 749, 000	21	0. 8	57	2. 0	78	2. 8
1958-----	2, 778, 000	35	1. 2	38	1. 4	73	2. 6
1959-----	2, 743, 400	265	9. 7	193	7. 0	458	16. 7
1960-----	2, 757, 500	6	. 2	19	. 7	25	. 9
1961-----	2, 766, 000	10	. 4	8	. 3	18	. 7
1962-----	2, 774, 000	4	. 1	3	. 1	7	. 2

¹ Populations of intercensus years are interpolated from the 1960 census.

The rates for paralytic and nonparalytic cases were almost equal. During 1959 a total of 458 cases was reported. The incidence of paralytic cases was one-third more than that of nonparalytic cases. The following year, 19 nonparalytic and 6 paralytic cases were reported. The number of reported cases declined to 18 in 1961 and to 7 in 1962. In both years the rates for paralytic and nonparalytic cases were almost equal.

The distribution of enteroviruses isolated from paralytic and nonparalytic patients is given in table 2, and the types of viruses isolated, in table 3. In 1957, virus studies were performed on 47 patients, 45 of whom were diagnosed as nonparalytic and 2 as paralytic. Fifteen viruses were isolated from the nonparalytic patients: two were polioviruses, one type II and one type III, and two were ECHO virus type 9. Coxsackie virus was recovered from 11 patients: 1 type A9, 3 type B3, and 7 type

B5. Specimens from the two paralytic patients were negative.

In 1958, specimens were available from 5 paralytic and 85 nonparalytic patients. Viruses were recovered from 24 patients. Of the viruses recovered from the five patients with a clinical diagnosis of paralytic poliomyelitis, four were poliovirus type I and one was ECHO virus type 9. Of the viruses recovered from the 19 nonparalytic patients, 9 were polioviruses: 5 type I and 4 type III. Other identifications were six Coxsackie virus (four type B2, one type B3, and one type B5) and two ECHO virus type 9. Two were unidentified.

Specimens from a total of 315 persons were tested for viruses in 1959. This group included 161 persons in a special study of the Des Moines epidemic. Polioviruses were recovered from 134 (72 percent) of 185 paralytic patients: 6 were type III, and the remainder (96 percent) were type I. In addition, one Cox-

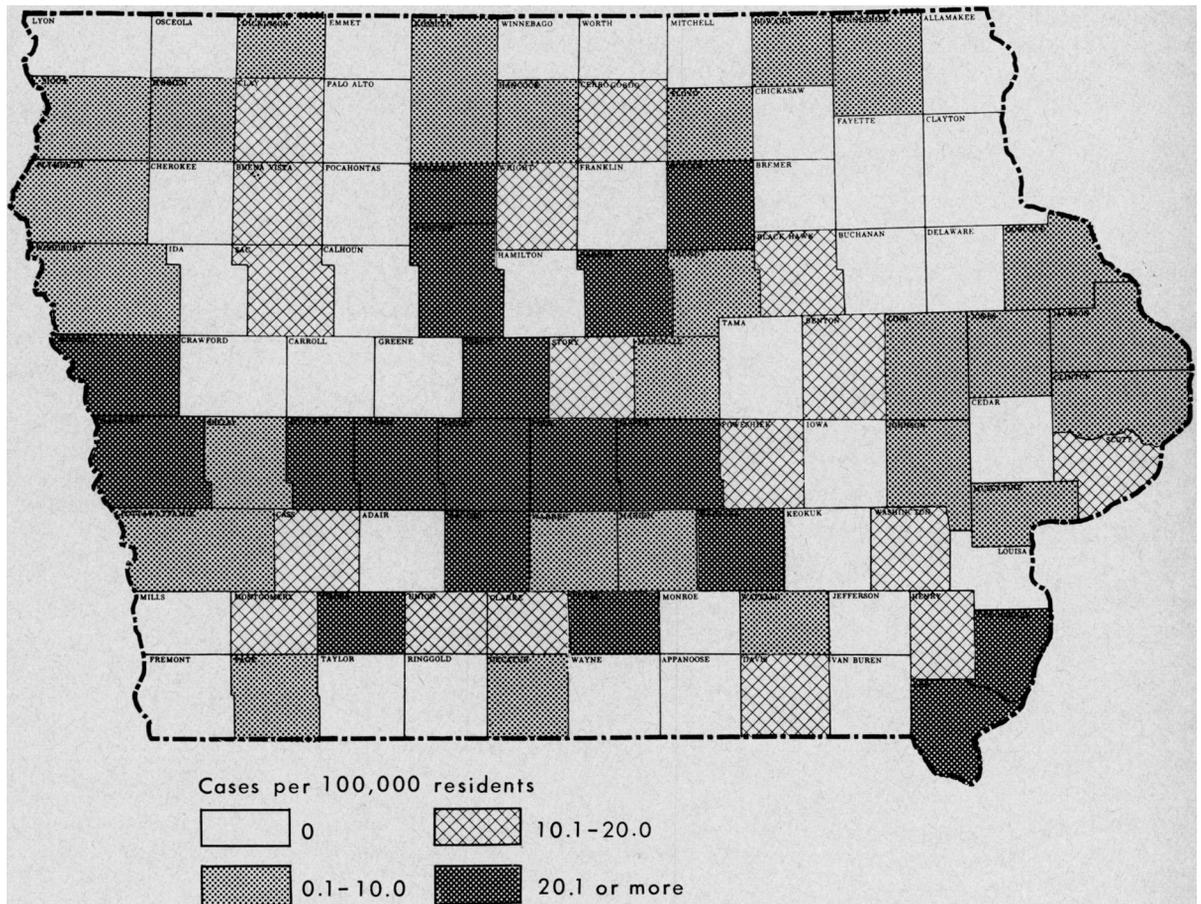
Table 2. Enterovirus isolations from paralytic and nonparalytic patients, by year, Iowa, 1957-62

Year	Paralytic patients				Nonparalytic patients				
	Number tested	Polio-virus	Cox-sackie	ECHO	Number tested	Polio-virus	Cox-sackie	ECHO	Uniden-tified
1957.....	2	0	0	0	45	2	11	2	0
1958.....	5	4	0	1	85	9	6	2	2
1959.....	185	134	1	0	130	37	3	9	4
1960.....	1	0	0	0	39	4	2	1	1
1961.....	0	0	0	0	248	0	74	3	8
1962.....	2	1	0	0	38	1	5	6	3
Total.....	195	139	1	1	585	53	101	23	18

Table 3. Types of enteroviruses recovered from patients with poliomyelitis and poliomyelitis-like illness, by year, Iowa, 1957-62

Year	Poliovirus			Coxsackie virus						ECHO virus								Uniden-tified	Total			
	I	II	III	A9	B2	B3	B4	B5	B6	1	4	7	9	10	11	14	18					
1957.....	0	1	1	1	0	3	0	7	0	0	0	0	2	0	0	0	0	0	0	0	0	15
1958.....	9	0	4	0	4	1	0	1	0	0	0	0	3	0	0	0	0	0	0	0	2	24
1959.....	164	0	7	0	4	0	0	0	0	0	2	3	0	0	1	3	0	0	0	0	4	188
1960.....	3	0	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	8
1961.....	0	0	0	2	9	0	1	62	0	1	0	0	1	1	0	0	0	0	0	0	8	85
1962.....	1	1	0	3	0	2	0	0	0	0	0	0	2	0	1	1	2	0	0	3	16	
Total.....	177	2	13	7	17	6	1	70	1	1	2	3	9	1	1	2	5	0	0	18	336	

Distribution of reported cases of poliomyelitis and poliomyelitis-like disease, by county attack rate, Iowa, 1959



sackie virus type B2 was isolated. The viruses of nonparalytic patients were more varied, but type I poliovirus was still the predominant agent. Poliovirus type I was recovered from 36 patients and type III from 1 patient. Three viruses were identified as Coxsackie B2. Nine ECHO viruses were identified: two type 4, three type 7, one type 14, and three type 18. Four viruses from the nonparalytic patients were unidentified.

In 1959 poliovirus infection was widespread throughout the State. The extent of the infection is reflected by the distribution of reported cases by county (see map).

Eighteen counties had attack rates of 20 or more cases per 100,000 population (table 4). Of the 314 cases reported in these counties, specimens were available from 250 patients. Viruses were recovered from 152 (61 percent) of the

patients. Seventeen counties had attack rates between 10 and 20. Viruses were recovered from 20 (55 percent) of 36 persons sampled. Twenty-six counties had an attack rate of less than 10. Viruses were isolated from 16 (55 percent) of 29 persons. No cases were reported from 38 counties.

Specimens from one paralytic patient were tested in 1960, but no virus was found. Eight viruses were isolated from 39 nonparalytic patients; 3 were identified as poliovirus type I, 1 as poliovirus type III, 1 as Coxsackie virus A9, 1 as Coxsackie virus B6, and 1 as ECHO virus type 9. One was unidentified.

No specimens from paralytic patients were tested in 1961. The nonparalytic cases reported included 198 persons sampled during an outbreak of aseptic meningitis and febrile illness in Fort Dodge. Specimens were obtained

from 248 persons. Viruses were isolated from 85 persons. Coxsackie virus B5 was the predominating agent. The following types of virus were recovered: 2 Coxsackie A9, 9 Coxsackie B2, 1 Coxsackie B4, 62 Coxsackie B5, and 1 each of ECHO virus types 1, 9, and 10 (reovirus type 1). Eight viruses were unidentified.

Poliovirus type I was recovered from one of two paralytic patients in 1962. Fifteen viruses were obtained from 38 nonparalytic patients. All but one were nonpolioviruses. The one poliovirus was identified as type II. The remaining viruses consisted of three Coxsackie A9, two Coxsackie B3, two ECHO 9, one ECHO 11, one ECHO 14, and two ECHO 18. Three viruses were unidentified.

The specific types of enteroviruses isolated from paralytic and nonparalytic patients during the 6-year period are summarized in table 5. This summary shows that poliovirus type I was the predominant cause of paralytic disease from 1957 through 1962. Of 195 paralytic patients sampled, enteroviruses were recovered from 141; 139 specimens (72 percent) yielded poliovirus: 133 type I and 6 type III. Only one each of the viruses Coxsackie B2 and ECHO 9 were isolated from the paralytic patients.

Enteroviruses were isolated from 195 (33 percent) of 585 nonparalytic patients. At least 17 different serotypes were identified. Only 53 (27 percent) of the 195 viruses were identified as poliovirus: 44 type I, 2 type II, and 7 type III. The remaining 142 isolates consisted of 101 Coxsackie viruses, 23 ECHO viruses, and 18 unidentified agents. Of the 101

Table 5. Types of enteroviruses isolated from paralytic and nonparalytic patients, Iowa, 1957-62

Type of virus	Paralytic patient	Non-paralytic patient	Total
Poliovirus:			
I.....	133	44	177
II.....	0	2	2
III.....	6	7	13
Total.....	139	53	192
Coxsackie virus:			
A9.....	0	7	7
B2.....	1	16	17
B3.....	0	6	6
B4.....	0	1	1
B5.....	0	70	70
B6.....	0	1	1
Total.....	1	101	102
ECHO virus:			
1.....	0	1	1
4.....	0	2	2
7.....	0	3	3
9.....	1	8	9
10 ¹	0	1	1
11.....	0	1	1
14.....	0	2	2
18.....	0	5	5
Total.....	1	23	24
Unidentified.....	0	18	18
Total.....	141	195	336

¹ Reovirus type 1.

Coxsackie viruses, 70 were type B5. This serotype was recovered mostly from patients with aseptic meningitis and febrile illness during an outbreak in Fort Dodge in 1961. The other Coxsackie types included: 7 of A9, 16 of B2, 6 of B3, and 1 each of B4 and B6. The 23 ECHO viruses included types 1, 4, 7, 9, 10, 11,

Table 4. Distribution of reported cases of poliomyelitis and poliomyelitis-like disease, and frequency of enterovirus isolations, by attack rates, Iowa, 1959

Attack rate per 100,000	Number of counties	Population ¹	Number of cases		
			Reported	Sampled	Enteroviruses isolated
More than 20.....	18	640, 750	314	250	152
10-20.....	17	560, 750	81	36	20
Less than 10.....	26	994, 040	63	29	16
None.....	38	547, 880	0	0	0
Total.....	99	2, 743, 420	458	315	188

¹ Population figures are interpolated from 1960 census.

14, and 18. Type 9 was the most common ECHO virus identified (table 5).

The isolation of ECHO virus type 18 from five patients with aseptic meningitis is of particular interest since few recoveries of this serotype from persons with aseptic meningitis have been recorded. Three of the patients, from separate families, were studied during the 1959 poliomyelitis epidemic in Polk County. In two families, some of the siblings had a similar illness. Serum samples from the three patients were not available for antibody studies.

The other two isolates of ECHO virus type 18 were obtained in 1962 from two female siblings who lived in Dallas County. The 6-year-old child became ill on July 29, and her 4-year-old sister on August 3. The diagnosis for both was aseptic meningitis. Stool and throat-gargle specimens were collected on August 6. Virus was isolated only from the stools. Acute and convalescent serum samples from the younger child were tested for ECHO 18 neutralizing antibodies. Antibodies were not detected in the acute serum at a dilution of 1:8 but were found in the convalescent serum at a dilution of 1:128.

Discussion

Surveillance of enterovirus infections for the 6-year study period 1957-62 revealed two major outbreaks, one caused by poliovirus type I and the other by Coxsackie virus B5. An extensive study of the poliomyelitis type I outbreak was conducted in Des Moines. Specimens submitted from other areas of Iowa indicated that this virus was widely disseminated throughout the State. Infection by Coxsackie virus B5 in 1961, however, was not as widely disseminated, although only a few specimens were submitted for study from areas other than Fort Dodge. Although various enteroviruses were recovered during the other study years, Coxsackie virus B5 was most frequently recovered in 1957 and poliovirus type I in 1958.

The surveillance program established in the eight selected areas of Iowa provided some information on the occurrence, distribution, and types of enterovirus infections in the State. It should be noted, however, that specimens were

routinely obtained only from patients who had an illness simulating poliomyelitis, and only a small percentage of the patients seen by physicians were sampled. Therefore, the enteroviruses that were isolated reflected only those most commonly causing poliomyelitis and poliomyelitis-like illnesses. Since the specimens were not tested in suckling mice, the occurrence of Coxsackie A virus in the population could not be determined.

The data obtained from this study provided additional information on the etiological relation of ECHO virus type 18 to aseptic meningitis. This virus was first isolated during the summer of 1955 by Ramos-Alvarez and Sabin (8) from a child with diarrhea. In 1956 Eichenwald and co-workers (9) studied an epidemic of diarrhea in a premature infant nursery and etiologically associated this virus with the outbreak. Recoveries of ECHO virus type 18 from the blood and stool specimens and throat washings of an infant who had a febrile illness with exanthem and from the stool specimen of an infant with fever, hematemesis, and coma have also been reported (10, 11).

ECHO virus type 18 had been recovered previously from only three patients with central nervous system disease (12-14). The diagnosis for two was aseptic meningitis and for one, paralytic poliomyelitis. In our study, the virus was recovered from five patients with aseptic meningitis, three in 1959 and two in 1962.

Summary

A surveillance study of enterovirus infections was conducted in Iowa from 1957 through 1962 by the Kansas City (Kans.) Field Station, Communicable Disease Center, Public Health Service, and the Iowa State Department of Health, Des Moines. During this 6-year period, two major outbreaks occurred, one caused by poliovirus type I and the other by Coxsackie virus type B5.

A total of 141 enteroviruses were isolated from 195 paralytic patients: 139 were polioviruses, 1 was ECHO virus type 9, and 1 was Coxsackie virus type B2. From 585 non-paralytic patients, 195 enteroviruses were isolated: 53 polioviruses, 23 ECHO viruses, and

101 Coxsackie viruses. Eighteen agents were unidentified.

Type I was the most common poliovirus and type B5 the most common Coxsackie virus isolated. Among the ECHO viruses, type 9 was most frequently isolated. Of particular interest is the isolation of ECHO virus type 18 from five patients with aseptic meningitis.

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Training Grants in Solid Wastes Technology

Awards totaling nearly \$150,000 were granted by the Office of Solid Wastes of the Public Health Service to Drexel Institute of Technology, Philadelphia, Pa., \$39,039; Georgia Institute of Technology, Atlanta, \$33,254; University of Michigan, Ann Arbor, \$40,129; and University of Texas, Austin, \$37,555, to train graduate engineers in solid wastes disposal technology.

The grants will make it possible for the institutions to offer curriculums for master of science degrees in chemical, civil, industrial, and sanitary engineering with emphasis on solid waste technology, raising the number of U.S. universities offering graduate engineering courses in solid wastes to six. Each grantee institution will add courses, laboratory study, seminars, and field exercises to basic solid waste courses.